

Certification date: 5 Nov 2017

ANALYSIS CERTIFICATE

Lot#: CyHum17017

PRODUCT DESCRIPTION

Reference: HuMC

Product: Human Hepatocytes

Category: Cryopreserved Plateable

Isolation date: 20 June 2017

Initial Isolation Viability (%): 82%

Storage conditions: Storage conditions: -196°C

DONOR DEMOGRAPHICS

| Species | Sex | Race | Age | BMI | Smoker | Alcohol Use | Drug Use |
|---------|------|-----------|-----|-----|--------|-------------|----------|
| Human | Male | Caucasian | 55 | 24 | No | No | No |

| Pathology | Medication | Serological Data |
|-------------------|------------|---|
| Colorectal cancer | N/A | Negative tested less than 3 months before operation |

Patient informed consent was obtained. The donor was serologically tested negative for following infectious diseases: HIV, Hepatitis B and C. Donor medical history were also examined prior to accepting this donor.

For in vitro use only, not to be used for clinical application. Products distributed by Cytes Biotechnologies may contain human material that should be treated as potentially hazardous.

CHARACTERIZATION FOR SUSPENSION CELLS

Lot#: CyHum17017

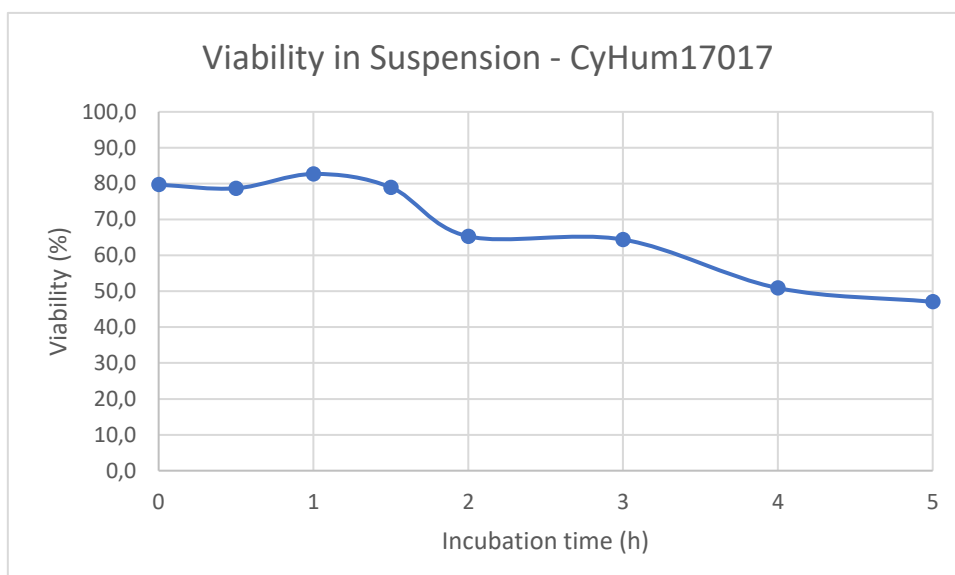
Post Thaw Lot information

Number of viable cells/vial: $6.0 \cdot 10^6$

Post-thaw viability (%): 79.7

Human hepatocytes were thawed according to Cytes Biotechnologies protocol. The post-thawing yield and viability (trypan blue exclusion assay) of hepatocytes were assessed after a purification process.

| Time (h) | 0 | 0.5 | 1 | 1.5 | 2 | 3 | 4 | 5 |
|----------------------|------|------|------|------|------|------|------|------|
| Viability (%) | 79.7 | 78.7 | 82.7 | 78.9 | 65.3 | 64.4 | 50.9 | 47.1 |



Resuspended human hepatocytes suspension ($0.5 \cdot 10^6$ cells in 0.5 ml medium) from the post-thaw assessment were incubated up to 5 h at 37°C. The assay was performed in 2 ml round-bottom tubes under shaking conditions (1000 rpm) using Eppendorf Thermomixer C. In the first two hours, samples were taken at every 30 min, after 2 h samples were taken at every 60 min. At each time point the viability of cells was calculated.

ENZYME ACTIVITY IN SUSPENSION CELLS

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- **PHASE I: CYP ACTIVITIES EXPRESSED IN pmol/min/mg protein (mean ± SD)**

| Induction (Specific Activity) | |
|-------------------------------|--|
| Isoforms | Induced Activity (pmol/min/mg protein) |
| CYP1A2 | 149.54 ± 8.72 |
| CYP2B6 | 77.40 ± 0.01 |
| CYP3A4/5 | 46.96 ± 0.70 |

Cryopreserved human hepatocytes in suspension culture (0.5×10^6 cells in 0.5 ml medium) were incubated with specific substrates (see table below) for 30 min at 37 °C for determination of CYP activities. The assay was performed in 2 ml round-bottom tubes under shaking conditions (1.000 rpm) in Eppendorf Thermomixer C. Metabolites were quantified by LC-MS and normalized to protein content. The substrates were applied as cocktail for simultaneous assessment of CYP 450 activity. Results are expressed in pmol/mg*min.

Table 1. Substrates Phase I

| Enzyme | Probe Substrate | Concentration (µM) | Incubation Time (min) | Metabolite |
|----------|-----------------|--------------------|-----------------------|--------------------|
| CYP1A2 | Phenacetin | | 30 | Acetaminophen |
| CYP2B6 | Bupropion | | 30 | Hydroxybupropion |
| CYP3A4/5 | Midazolam | | 30 | 1-Hydroxymidazolam |

Signed:



Quality Manager
Unit for Research
Cytes Biotechnologies

